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EXPERIMENTAL RESEARCH ON CLARIFYING CARCINOGENEITY OF SHALE OIL SOOT

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EXPERIMENTAL RESEARCH ON CLARIFYING CARCINOGENEITY OF SHALE OIL SOOT
[Eksperimental'nyye issledovaniya po vyyasneniya kantserogennosti slantsevoy sazhi]

by

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At the present time, the environment is generally recognized from the point of view of etiological importance of carcinogens in the genesis of lung cancer in man. All of the growing studies on illness from cancer and its location involves contamination of the air which man breathes along with the products of incomplete combustion of fuel: soot, tarry substances in various heating systems, exhaust gases of vehicles, tobacco smoke, and also the effect of ionizing radiation [8,10].

Most chemical carcinogens observed in the contaminants of the air atmosphere belong to a group of polycyclic aromatic hydrocarbons including 3,4-benzpyrene which is considered the most important.

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It is appropriate to mention that the first physical and chemical studies of carcinogenic substances, in particular, 3,4-benzpyrene, in solid contaminants of the air atmosphere of cities began at practically the same time in the Soviet Union and in England in 1948--49 [10]. From this time on, carcinogens in various quantities were detected in the contaminated air of almost all industrial cities of the world [7,10].

Fuel shale oils are low-calorie fuel which in comparison with other types of solid fuel are distinguished by a large content of mineral compounds, and its fuel part is very rich in volatile components [1]. As a result of the abundance of mineral compounds during combustion of shale oils, a great deal of ash (45--75% of the weight of the shale) forms which, preventing the supply of oxygen to the fuel part of the shale, creates conditions for its incomplete combustion. Therefore, when burning shale in ordinary fire grate furnaces, smoke forms which contains a great deal of soot and tarry substances.

Considering the circumstance mentioned, it seemed pertinent to clarify in biological tests the carcinogenicity first of the soot which forms during combustion of kukersit fuel shales (solid fuel) and also the soot of fuel oil (mazut) obtained during the thermal processing of fuel shales.

The results of chronic tests made in 1955-56 by applying benzene extract of the soot of shale solid fuel to the skin of backs of 100 white mice clearly showed its carcinogenicity. Skin tumors developed in 58 mice (78%) of the 74 animals who survived the first 5 months of the test. Then, 36 mice were diagnosed as having skin cancer [2,3]. Data from spectral-fluorescent analysis done by P.P. Dikun showed the

presence of 3,4-benzpyrene in soot as 0.0014% and the benzene extract in it--0.04 mg per 1 mℓ.

Carcinogenic activity was observed by us in the soot of shale mazut [3]. In this series of tests, made in 1959--60, 188 mice were used. The carcinogenicity of this type of soot was less well expressed than the soot of the solid fuel. Thus, tumors of the skin occurred at the location of application in 26 mice of 141 surviving 5 months after the beginning of the test; most of the tumors which occurred were benign and some of them were found to reverse themselves after the 15-time applications were stopped. In all, 9 mice were observed to have tumors (6.4%) and in 3 cases, skin cancer was observed.

The carcinogenic activity of soot and other atmospheric contaminants in the biological test was greater the higher was the content in them of 3,4-benzpyrene [10]. According to the data of fluorescent-spectral analysis, benzene extract studied by us in the soot of shale mazut shown to be less active in the biological test, contained even more, (0.12%) 3,4-benzpyrene than the benzene extract of soot of solid fuel shale, in 1 mℓ of which there was 0.04 mg 3,4-benzpyrene. In all probability, in the soot of the shale mazut, there are some kind of unknown components which decreased its biological effect.

The results of the tests mentioned above carried out on mice showed that the soot of fuel shales is carcinogenic. Subsequently, the question arose as to how shale soot or its extract of tarry substances induces tumors in the lungs.

In 1959-60 when we began our research, according to bibliographical data [8,9] obtaining experimental tumors of the lung by introducing carcinogenic

substances was done on mice, but the tumors induced in them in most cases were of the adenomata type which according to morphology, histogenesis, course, localization and other factors are considerably different from lung cancer in humans. There was data on the extreme rarity of spontaneous new formations, in particular, of epithelial tumors of the lung in the laboratory rats. As concerns individual tumors of the lung in rats, in 1960 there was information on the possibility of systematically obtaining experimental lung cancer in these animals only by introducing radioactive substances into their lungs. There was not yet a suitable experimental model for obtaining lung cancer with chemical carcinogens. Only a few years later, an adequate experimental model was created for lung cancer of man using powerful pure carcinogens introduced into rats or hamsters--3,4-benzpyrene, 9,10-dimethyl-1,2-benzanthracene, 3-methylcholanthrene and others [8].

Inasmuch as we carried out these tests on inducing lung tumors on miscellaneous white rats of both sexes (separate nurseries of "Rappolovo" AMN SSSR [Akademiya meditsinskikh nauk SSSR, Academy of Medical Sciences of the USSR]).

The first problem was to give an oncological character to the control animal, not subjected to any kind of experimental effect. There were 150 animals (80 males and 70 females) in this control group with age 1.5--2.5 years, and they were maintained on an ordinary food ration in the vivarium of the Institute. Spontaneous tumors were observed in all the 12 rats (8 female and 4 male) and there were 2 cases of primary reticulosarcoma of the lungs. Spontaneous epithelial tumors of the lungs were not encountered.

Pre-tumor changes in the lungs of the "middle-aged" rats were a rare phenomenon. In our material, they were observed in 8 cases (out of 150 rats) and were found either in the form of non-uniform hyperplasia of bronchial epithelia (according to L.M. Shabad--the first stage of the tumor process) or more rarely--in the form of separate adenomatic proliferates (second stage).

It was interesting to establish if breathing shale soot in volatile ashes causes pre-blastomogenic changes more often than in the control group. Lungs of rats were studied which during the test were used for studying the pneumococcus danger of a given mixture of dust; during 9 months they were subjected to dust inhalation in the chamber and then remained under observation for up to 11 months. The concentration of dust in the chamber in the first 3 months was 965 mg/m^3, and from the fourth month on the inhalation test--365 mg/m^3; the dust contained 0.0014% of 3,4-benzpyrene. Pre-tumor changes (mainly in the form of focal proliferates) were observed considerably more often ($p<0.01$) than in the control (in 9 rats of the 33 animals who survived the entire period of the experiment). Moreover, in one rat in this experiment there was a lung tumor--a malignant epidermal cyst.

The soot of shale fuel and the benzene extract of tarry substances manufactured from it with a content of 3,4-benzpyrene in them can be considered as so-called weak carcinogenic substances. Therefore, for clarifying the possible blastomogenic effect on the lungs of rats we decided to combine their effect with chemical or physical factors which have a certain capacity for producing tumors in the lungs in this type of animal (urethane, ionizing radiation) or by making

modifications--strengthening the effect of the activity of the carcinogenic substance (Tween 40 solution).

The results of the first series of tests in 1960-1962 on 432 rats in which there was intratracheal introduction of certain types of shale soot or tarry substances prepared from them combined with long term peroral introduction of ethylurethane, were the following. In a test with long term peroral introduction of urethane in white rats (3 times a week, 50 mg for up to 16 months from the beginning of the test until the death of the animal) we successfully induced epitheliomas in the lungs only in a few cases--in all, in 4 rats out of the 46 animals who lived for the entire test period. Then there were 2 cases of alveolar adenomata and 2 cases of keratosic epidermoidal tumors; of these one was benign in the form of an epidermoidal cyst, and the other--a small focus of epidermoidal cancer with growth in the wall of the lobar branch of the lung artery.

Intratracheal introduction of shale mazut soot which contains 0.02% 3,4-benzpyrene or 2 types of solid fuel shale soot (soot with an admixture of volatile ash and content 0.00041% 3,4-benzpyrene) and soot containing 0.0107% 3,4-benzpyrene, did not have a noticeable effect on the frequency of lung tumors induced by urethane (with repeated (10 times) intratracheal introduction of a saturated oil solution of tarry substances extracted from these types of soot (single dose 0.1 mℓ) in rats which receive urethane, the frequency of tumors of the lungs was higher than with introduction only of urethane, but in comparison with the χ^2-test, the difference was not significant. The introduction of only tarry substances of soot dissolved in peach oil,

in this series of tests did not cause tumors in the lungs; in part of the animals studied only pre-tumor changes of bronchial and alveolar epithelia developed.

The results of the tests of this series agree with the bibliographic data on unsuccessful tests to cause the growth of tumors of the lung by intratracheal introduction of coal tars, various types of soot and non-radioactive dust [8]. On the other hand, the results of our control tests with peroral introduction of urethane showed that this substance, according to bibliographic data possessing only pulmonary blastomogenic effects for the development of strains of laboratory mice [8,9] has an average carcinogenic effect on the lungs of rats which essentially is not increased by combining the effect of urethane with the effect of another blastomogenic agent--intratracheal introduction of shale soot or tarry substances from the soot.

In further tests, our main attention was directed to creating conditions for penetration and depositing of the carcinogenic substances being studied by us in the lung tissue of the test rats. From literature it was established that non-ionizing surface-active lipophilic-hydrophilic substances (detergents) of the Tween type are physically modifying factors capable of penetration of the carcinogen and having itself a potential effect of small doses of carcinogen. A similar effect was observed by K. Setälä [13] and others in many tests for inducing tumors of the skin in mice, Herrold and Dunham [11]--with the induction of 3,4-benzpyrene of tracheobronchial tumors in Syrian rabbits and Rigdon [12]--inducing tumors of the lungs in Peking ducks.

Relying on the data of the bibliography, in 1963-65 we carried out

a basic series of tests in which tarry substances extracted from the soot of shale of solid fuel were introduced (with a content in the soot of 0.0107% 3,4-benzpyrene) in the test rats intratrachealy in 2 different suspension media [6].

The test rats were divided into 2 groups. The animals of group I (125 rats) were injected 10 times at 1-week intervals with 0.1 mℓ suspension of tarry substances of soot in a 12.5% solution of Tween 40 solvent; the animals of group II (100 white rats) received the dose in a suspension of the mixture in peach oil. Both mixtures were prepared calculating 100 mg of tarry substance of soot for 1 mℓ of Tween 40 solvent or oil. Part of the animals from both test groups, after the intratracheal injections were stopped in a 5--6 month tests (with age 7--8 months) were subjected to a 1-time general irradiation with X-ray radiation, dose 400 r.

The control animals were: 1) rats who received repeated intratracheal injections only of Tween solution (39 animals); 2) 27 white rats who were injected only with vegetable oil. Epithelial tumors of the lungs were not observed in these control animals or in the "middle-aged" control rats; 3) of the 34 control rats subjected to general radiation with X-rays, 2 animals had adenomata tumors in the lungs.

An analysis of the results of this series of tests make it clear that ionizing radiation of rats who had previously received the intratracheal injections described above in vegetable oil or in a suspension of Tween 40, were essentially not affected either by the general occurrence of tumors or the frequency and character of lung tumors (see Table 1) ($\chi^2 = 1.024$ $0.7>p>0.5$). The responsive reaction of the lungs from the oncological aspects depended only on the character of the medium in which

TABLE NO. 1 LUNG TUMORS IN TEST AND CONTROL RATS

Groups of Animals According to Experimental Effect	Number of Rats Used in the Tests	Number of Rats Studied Microscopically	Number of Rats Who Lived 5 Months from the Beginning of the Test*	Number of Rats with Lung Tumors					
				Benign			Malignant		
				Adenoma	Epidermal Tumor	Tumor with Mesenchyme Origin	Cancer	Sarcoma	Total Number of Cases
Test Groups									
I. Tarry substances of shale soot in Tween 40 (12.5%)									
Total rats	125	84	70	2	23	--	8	1	34
a) without irradiation		46	32	1	10	--	5	1	17
b) with irradiation (400 r)		38	38	1	13	--	3	--	17
II. Tarry substances of shale soot in peach oil									
Total rats	100	63	57	2	1	--	--	1	3
a) Without irradiation		32	26	2	--	--	--	1	2
b) With irradiation (400 r)		31	31	--	1	--	--	--	1
III. 3,4-benzpyrene in peach oil	54	52	51	1	1	1	--	6	9
IV. 3,4-benzpyrene in polyglucin	54	53	46	5**	2	1	2	3***	12
V. 3,4-benzpyrene in 1% solution Tween 80	56	44	39	4	13	--	3	2"	21

TABLE NO. 1 LUNG TUMORS IN TEST AND CONTROL RATS (Continued)

Groups of Animals According to Experimental Effect	Number of Rats Used in the Tests	Number of Rats Studied Microscopically	Number of Rats Who Lived 5 Months from the Beginning of the Test*	Number of Rats with Lung Tumors					
				Benign				Malignant	
				Adenoma	Epidermal Tumor	Tumor with Mesenchyme Origin	Cancer	Scarcoma	Total Number of Cases
VI. 3,4-benzpyrene in 12.5% solution Tween 40	104	71	55	3"	15	3*"	9	--	27
Control Groups									
VII. Peach oil	31	27	27	--	--	--	--	1	1
VIII. Tween 40 or 80	49	39	35	--	--	--	--	--	--
IX. Control without intratracheal injection	150	150	150	--	--	--	--	2	2

ANNOTATION:
*) or the number of rats who were alive at the moment of the appearance of the first tumor (if the tumor of the lung is found before the 5 month period)
**) of these, one adenoma and 4 cases of multi-focal adenomata
***) of these in 1 rat there was lung and multi-focal adenomata
") of these, in 1 rat there was epidemoidal tumor of the lung
*") of these, in 2 rats there were epidemoidal tumors of the lung.

the tarry substances were suspended or dissolved.

There is interest in the fact that progressively the growth of bronchial type epidermoid blastomata are only in rats of the first test group who received tarry substances in a suspension of Tween 40. In this group, the 70 rats alive at the time of the appearance of the first lung tumor, 33 animals were found, besides one case of adenoma, to have epidermoidal tumors (keratosic flat-cell papillomata of the bronchus) or epidermoidal cysts, often multiple and in 8 rats--epidermoidal cancer of the lung (Figures 1 a, b, and 2 a, b), metastases in the kidney of 2 rats).

Of the rats in the II test group who received injections of tarry substances of soot in vegetable oil, only in 3 of the 57 who lived 5 months from the beginning of the test, were there isolated epithelial tumors in the lungs (in 2 rats--adenomata, in 1--epidermoidal cyst).

Thus, the results of this series of our tests support the possibility of developing lung tumors due to the effect of tarry substances from soot of fuel shales, that is, from a carcinogenic substance weak in content of 3,4-benzpyrene. On the other hand, the results of present experiments give us information on the important role of the character of the suspension medium when inducing lung tumors in white rats by repeated introduction of a carcinogenic substance--tarry substances from the soot of fuel shales.

The fact that the physical and chemical character of the liquid medium used for suspending the carcinogen has great importance when obtaining tumors of the lungs in rats; once again the results of the succeeding series of tests supported this, those made in 1963-65 on white rats with pure 3,4-benzpyrene (Table 1). This carcinogen was

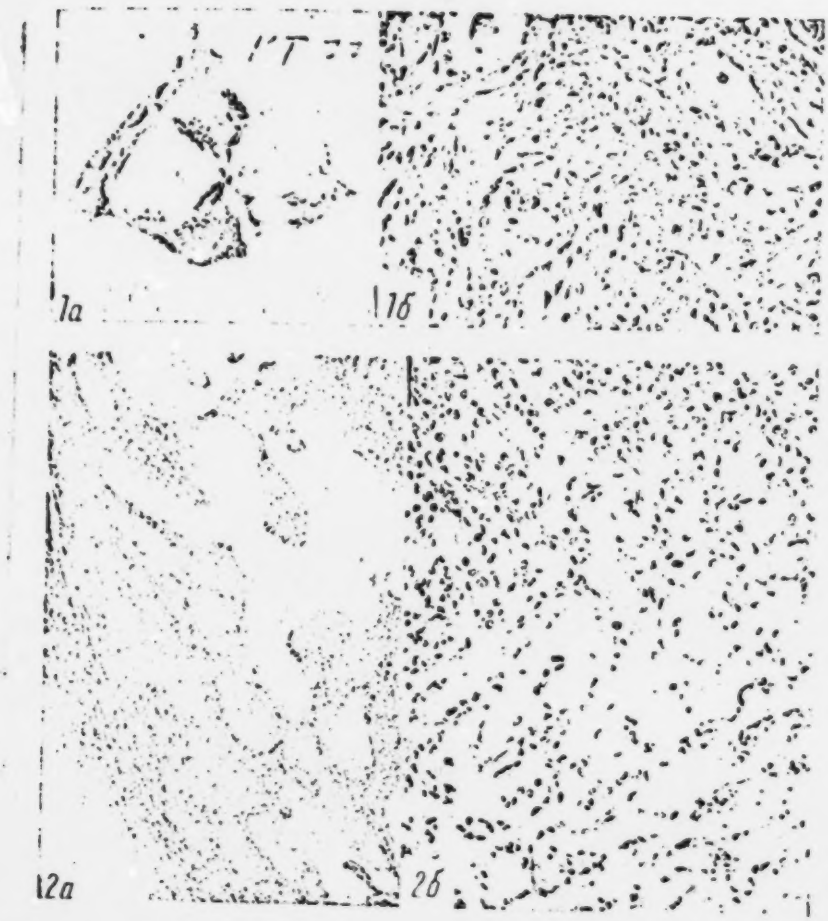

Figure 1. Epidermoidal Cancer of the Lung of a White Rat Who Died after 17 Months and 23 Days from the First Intratracheal Injection of Tarry Substances from Shale Soot in a Suspension of Tween 40. Figure 1 a--Macro-photo of the lungs with nodular tumor. Figure 1 b-Keratosic epidermoidal cancer. Hemotoxylin and eosin.

Figure 2. Epidermoidal Cancer of the Lung of a White Rat Who Died after 13 Months after the First Injection of Tarry Substances from Shale Soot in a Suspension of Tween 40. Figure 2 a--A peripheral section of keratosic epidermoidal tumor. Figure 2 b--Another secion with non-keratosic growth of cancer ephithelia and scirrhomous stroma. Coloration according to van Gizon.

introduced intratracheally 3 times, in 5 mg, at monthly intervals between injections (in all 15 mg) in a suspension of one of the following liquids; 1) peach oil; 2) blood substitute polyglucin; 3) a 1% aqueous solution of Tween 80 and 4) 4.5% aqueous solution of Tween 40. The individual dose of each of these liquids for 5 mg of carcinogen was 0.2 mℓ. Epithelial, in particular epidermoidal, tumors of the lungs with significant frequency can be induced in rats under certain test conditions only using intratracheal instillation of 3,4-benzpyrene in a suspension of Tween 40 or 80 solutions. Then, the media of induced epidermoidal tumors of the lungs are encountered in all stages of progression of a given histological type of lung cancer. Many of these were characterized by non-invasion growth (benign keratosic epidermoidal epithelomata in the form of flat-celled papillomata of the bronchi, epidermoidal cysts in respiration tissue or on the smaller bronchi), and their degree of proliferation had significant variations. Epidermoidal individual cancers in the data of test conditions corresponded both to localized and to histological structure of epidermoidal cancer of humans.

The use of polyglucin as a suspension medium for 3,4-benzpyrene resulted in a lower frequency of formation of epithelial tumors in the lungs than did the use of Tween solutions, but more occurred than when vegetable oil was used for this.

At the present time, in the morphology laboratory of the Institute, research on experimental cancer of the lung in white rats (miscellaneous and Wistar breeds) continues in the direction of studying possible potential effects of certain dusts (dust of fuel shales and serpentine asbestos)

on lung carcinogenesis induced by 3,4-benzpyrene. The latter subject being worked on by R.A. Salk and A.I. Vysamyae is included in the plan for research work at the International Agency of VOZ [Vsemirnaya organizatsiya zdravookhraneniya, World Health Organization (WHO)] for studying cancer in Lyons (France) for 1970--1971.

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